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New generation of supramolecular mixtures: Characterization and solubilization studies

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► **To cite this version:**

Tracy El Achkar, Leila Moura, Tarek Moufawad, Steven Ruellan, Somenath Panda, et al.. New generation of supramolecular mixtures: Characterization and solubilization studies. *International Journal of Pharmaceutics*, 2020, 584, pp.119443. 10.1016/j.ijpharm.2020.119443 . hal-03102809

HAL Id: hal-03102809

<https://hal.science/hal-03102809>

Submitted on 22 Aug 2022

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22 **Abstract**

23 In this work, a series of novel low melting mixtures (LMM) based on cyclodextrins (CD)
24 and levulinic acid and inspired by the deep eutectic solvents (DES), were prepared.
25 These supramolecular mixtures are the first reported CD-based mixtures that are liquid
26 at room temperature. Density, viscosity and rheological measurements as well as
27 differential scanning calorimetry and thermogravimetric analysis were performed to
28 characterize these new LMM. Nuclear magnetic resonance (NMR) spectroscopy was
29 used to monitor their stability. Furthermore, their ability to solubilize *trans*-anethole (AN)
30 and related essentials oils were evaluated by static headspace-gas chromatography
31 (SH-GC), in comparison with water. AN was up to 1300 times more soluble in the CD-
32 based LMM than in water. Finally, multiple headspace extraction (MHE) was used to
33 monitor the release of AN from these LMM. After 10 extractions, 20 to 40% of AN was
34 released from the studied LMM, while 70% was released from water. The new CD-
35 based LMM have potential applications for solubilization and delivery of poorly soluble
36 drugs.

37

38 **Keywords:** cyclodextrins, static headspace-gas chromatography, *trans*-anethole,
39 essential oils, low melting mixtures.

40

41

42 **1. Introduction**

43 Cyclodextrins (CD) are non-toxic cyclic oligosaccharides obtained from the enzymatic
44 degradation of starch (Crini, 2014; Szejtli, 1998). Native CD, which are produced at an
45 industrial scale, consist of six (α -CD), seven (β -CD) or eight (γ -CD) α -(1 \rightarrow 4) linked D-
46 glucopyranose units. Due to their hydrophobic internal cavity and hydrophilic external
47 surface, these water-soluble molecules have been extensively used to encapsulate
48 hydrophobic and volatile compounds by forming host-guest inclusion complexes
49 (Ciobanu et al., 2013; Kfoury et al., 2019, 2015; Marques, 2010).

50 β -CD is the most studied and most frequently used CD, because of its cheapness and
51 availability. However, it is the least water-soluble of the three native CD. Therefore,
52 various β -CD derivatives have been synthesized and are commercially available. CD
53 and their derivatives have attracted interest for a wide range of applications such as
54 food, agrochemistry, environmental chemistry, catalysis, cosmetics and pharmaceuticals
55 (Crini et al., 2018; Kurkov and Loftsson, 2013; Landy et al., 2012; Legrand et al., 2009;
56 Loftsson and Masson, 2001; Nguyễn et al., 2017; Potier et al., 2012).

57 The discovery of deep eutectic solvents (DES) in 2003 was a turning point in the world
58 of green chemistry (Abbott et al., 2003). Since then the publications in this field have
59 increased exponentially. Being easy to prepare from cheap and widely available starting
60 materials, DES have attracted a lot of attention from researchers in the past few years
61 and have found applications in a wide range of domains: metal processing, synthesis,
62 electrochemistry, solubilization of gas and pollutants, extraction of bioactive compounds
63 and as drug solubilization vehicles (El Achkar et al., 2019; Li and Lee, 2016; Mbous et
64 al., 2017; Morrison et al., 2009; Moura et al., 2017; Zhang et al., 2012). These solvents

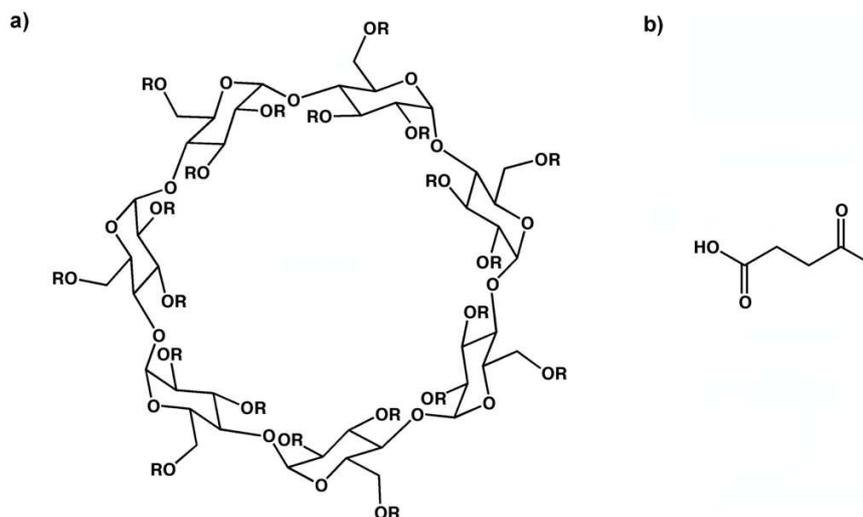
65 are obtained by mixing two or three compounds that are able to associate, mainly by the
66 formation of hydrogen bonds, at a particular molar ratio to form a clear homogenous
67 liquid with a lower melting point than that of the individual components (Zhang et al.,
68 2012). Among them, DES based on carbohydrates and other primary metabolites have
69 been described lately (Choi et al., 2011). These DES are called natural deep eutectic
70 solvents (NADES). In this respect, cyclodextrins (CD) are potential NADES-forming
71 compounds.

72 Only a few studies have investigated the combined use of CD and DES. Some studies
73 have evaluated the possibility of forming low melting mixtures (LMM) with CD and *N,N*-
74 dimethylurea (DMU) (Ferreira et al., 2015; Imperato et al., 2005; Jérôme et al., 2014). A
75 LMM was indeed obtained with various CD derivatives. Hydroformylation, Tsuji-Trost
76 and Diels–Alder reactions were efficiently performed in these new solvents with high
77 catalytic activity. However, the melting point of these LMM was superior to 80 °C,
78 limiting their scope of application. Thereafter, we have patented the use of DES for the
79 absorption of volatile organic compounds (VOC) and described the beneficial effect of
80 adding CD on VOC solubility in a choline chloride:urea (ChCl:U) DES (Fourmentin et al.,
81 2016). At the same time, a study showing that the solubility of the three native CD was
82 remarkably enhanced in the same DES was published (McCune et al., 2017). Moreover,
83 it was recently reported that no aggregation of β -CD occurred in ChCl:U up to
84 concentrations of 800 mg/mL (Triolo et al., 2020). Methyl- β -CD has also been
85 incorporated into aqueous solutions of a DES based on glycerol and glycine and
86 described to improve the extraction of polyphenols (Athanasiadis et al., 2018). Later, we
87 investigated the advantage of combining ChCl:U and CD to solubilize various volatile

88 compounds and demonstrated that CD maintain their host-guest properties in ChCl:U
89 (Di Pietro et al., 2019; Dugoni et al., 2019; Moufawad et al., 2019). Combinations of
90 DES and CD can potentially give rise to highly attractive materials; however, having a
91 solvent based on CD could be even more useful. To this end, we recently reported the
92 first supramolecular mixture based on randomly methylated- β -CD (RAMEB) and
93 levulinic acid and showed that RAMEB retained its inclusion properties (El Achkar et al.,
94 2020).

95 In the present study, three new supramolecular mixtures were prepared using new β -
96 CD derivatives (HP- β -CD, CRYSMEB and Captisol®) in addition to RAMEB as
97 hydrogen bond acceptors (HBA) with levulinic acid (Lev) as hydrogen bond donor
98 (HBD) (Figure 1). Density, viscosity and rheological measurements, differential
99 scanning calorimetry and thermal gravimetric analysis were carried out to characterize
100 these new liquid mixtures. Moreover, their stability was evaluated as a function of time
101 and temperature by NMR. Finally, their ability to solubilize trans-anethole (AN), star
102 anise and fennel essential oils was evaluated by static headspace-gas chromatography
103 (SH-GC) and multiple headspace extraction (MHE) was used to monitor the release of
104 AN from these mixtures.

105



106

107 Figure 1. Structures of the low melting mixtures constituents a) General structure of β -
 108 cyclodextrin derivatives: HP- β -CD (degree of substitution (DS) = 5.6), R= -H or -CH₂-
 109 CH(OH)-CH₃; RAMEB (DS = 12.9), R= -H or -CH₃; CRYSMEB (DS = 4.9), R= -H or -
 110 CH₃; Captisol® (DS = 6.5), R= -H or -(CH₂)₄-SO₃⁻ Na⁺; b) Levulinic acid.

111

112 2. Experimental Section

113 2.1. Materials

114 *trans*-Anethole [1-methoxy-4-(1-propenyl)-benzene] (99%) was purchased from Acros
 115 Organics, France. Levulinic acid (98%) was purchased from Sigma-Aldrich, France.
 116 Randomly methylated- β -CD (RAMEB, DS =12.9), low methylated- β -CD (CRYSMEB,
 117 DS = 4.9) and hydroxypropyl- β -CD (HPBCD, DS= 5.6) were provided by Roquette
 118 Frères (Lestrem, France). Sulfobutylether- β -CD (Captisol®, DS= 6.5) was provided by
 119 LIGAND Pharmaceuticals (San Diego, CA, USA). All compounds were used as
 120 received.

121 Essential oils of *Illicium verum* (star anise) and *Foeniculum vulgare* (fennel) were
 122 provided by Herbes et Traditions (Comines, France). The main constituents (>1%) of

123 the essential oils are: *trans*-anethole (91.07%) and limonene (3.28%) for star anise;
124 *trans*-anethole (75.30%), estragole (3.10%) and fenchone (2.10%) for fennel (data
125 provided by Herbes et Traditions).

126

127 2.2. Preparation of the supramolecular mixtures

128 The LMM were prepared by mixing the HBA and the HBD at a fixed molar ratio (Table
129 1). The mixtures were then stirred at 60 °C until the formation of a clear homogenous
130 liquid then cooled at room temperature. All the prepared mixtures were liquid at room
131 temperature. As CD possess a large number of HBA sites (35 sites for the native β -CD
132 and 35 to 55 sites for the modified β -CD), we used a large molar excess of levulinic
133 acid. The water content of all the prepared mixtures was determined using Karl Fisher
134 titration method (Mettler Toledo DL31) and was found to vary between 2.5 and 3.9 wt.%
135 (Table 1).

136

137 Table 1. Composition and water content of the prepared LLM.

CD:Lev mixture	HBA	HBD	HBA:HBD molar ratio	Water content (wt. %)
HPBCD:Lev	Hydroxypropyl- β -CD		1:32	2.7
RAMEB:Lev	Randomly methylated β -CD	Levulinic Acid	1:27	2.5
CRYSMEB:Lev	Low methylated β -CD		1:25	3.3
Captisol®:Lev	Sulfobutylether- β -CD		1:44	3.9

138

139 We should point out that our attempts to obtain liquid mixtures with the three native CDs
140 failed, which tends to emphasize that the density and flexibility of the hydrogen bond
141 network are probably key parameters for the formation of liquid mixtures. It seems that

142 the breakdown of hydrogen bonds from one glucose to another within CD molecules
143 could be a prerequisite, therefore limiting the preparation of such LMM to modified CDs.

144

145 *2.3. Stability studies of LMM by NMR*

146 In order to evaluate whether any esterification reaction occurred between the remaining
147 hydroxyl groups of the CD derivatives and levulinic acid, the supramolecular mixtures
148 were prepared at 30 °C and were subjected to gradual heating. Aliquots were withdrawn
149 at different times and temperatures, then diluted with DMSO-*d*₆, or CDCl₃, in the case of
150 Captisol®:Lev, and analyzed by NMR. The first aliquot of the mixture was taken when a
151 clear liquid was formed (*t*₀). The other aliquots were withdrawn at 30, 60, 80 and 100
152 °C. The mixtures were kept for 24 hours at the desired temperature prior to analysis. ¹³C
153 NMR experiments were recorded on a Bruker Avance III spectrometer operating at 400
154 MHz for the proton nucleus, equipped with a multinuclear z-gradient BBFO probe head.
155 The probe temperature was maintained at 30 °C and standard 5 mm NMR tubes were
156 used. ¹³C spectra were recorded with the following acquisition parameters: time domain
157 65 K with a digital resolution of 0.73 Hz, relaxation delay: 2 s and 1536 scans.

158

159 *2.4. Density and viscosity measurements*

160 Density measurements were carried out using a U-shaped vibrating-tube densimeter
161 (Anton Paar, model DMA 5000 M) operating in a static mode. The factory calibration
162 was used and verified before and after each measurement with air and tri-distilled
163 degassed water. The DMA 5000 densimeter performs an analysis with an estimated
164 uncertainty in density and temperature of ± 0.1 kg m⁻³ and ± 0.001 °C, respectively.

165 The viscosity was determined using a falling-ball-based microviscosimeter (Lovis 2000
166 M/ME from Anton Paar). The temperature was controlled to within 0.005 °C and
167 measured with an accuracy better than 0.02 °C. A capillary tube of 1.8 mm diameter,
168 previously calibrated as a function of temperature and angle of measurement with
169 reference oils, was used for the measurements. The overall uncertainty on the viscosity
170 was estimated to be 2%. All measurements were performed at atmospheric pressure
171 and at temperatures ranging between 30 and 60 °C, as previously described (Moufawad
172 et al., 2019).

173

174 *2.5. Rheological measurements*

175 Rheological measurements were performed with an AR-G2 controlled-stress rotational
176 rheometer (TA Instruments). Flow curves were obtained with an aluminum cone-plate
177 geometry (40 mm diameter, 1° cone angle, 28 µm truncation gap). A three-step shear
178 rate sweep was imposed after a 3-minute equilibration time: 1) increase of the shear
179 rate from 0.1 to 5000 s⁻¹ over 3 min (upwards curve), 2) peak hold at 5000 s⁻¹ during 1
180 min, 3) decrease of the shear rate from 5000 to 0.1 s⁻¹ over 3 min (downwards curve).
181 The temperature was maintained at 30 °C and controlled with a Peltier plate.
182 Measurements were performed in triplicate at least for each sample, to ensure
183 reproducibility. The statistical analysis was performed by calculating the standard
184 deviation from the three measurements or more made for each sample.

185

186 *2.6. Differential scanning calorimetry (DSC)*

187 DSC experiments were carried out using a Q1000 DSC (TA Instruments) with a
188 temperature range from -100 °C to 40 °C and at a thermal scanning rate of 5 °C.min⁻¹.
189 All the samples (HPBCD:Lev, RAMEB:Lev, CRYSMEB:Lev and Captisol®:Lev) were
190 encapsulated in aluminum pans (sample weight ~ 10-15 mg), sealed with hermetic lids
191 and analyzed. Experiments were performed under nitrogen flow (50 mL.min⁻¹).

192

193 *2.7. Thermal gravimetric analysis (TGA)*

194 TG measurements were performed with a TGA550 thermogravimetric analyzer (TA
195 Instruments). Samples were placed in an open platinum pan (100 µL) suspended in the
196 furnace. The initial weight of the sample was around 25-30 mg, and nitrogen was used
197 as the purge gas at a fixed flow of 20 mL.min⁻¹. The weight of material was recorded
198 during heating from room temperature to 600 °C at a heating rate of 10 °C.min⁻¹.

199

200 *2.8. Static Headspace-Gas Chromatography (SH-GC)*

201 AN, star anise and fennel essential oils (EO) were added to water or to the LMM placed
202 in 22 mL headspace glass vials. Vials were then sealed and thermostated at 30 °C
203 under stirring for 24 hours in order to reach equilibrium between liquid and gaseous
204 phases. Subsequently, 1 mL of the gaseous phase was withdrawn from the vial and
205 injected in the chromatographic column for analysis via a heated transfer line.

206 All experiments were carried out with an Agilent G1888 headspace sampler coupled
207 with a Perkin Elmer Autosystem XL gas chromatography equipped with a flame
208 ionization detector and a DB624 column using nitrogen as carrier gas. The GC column
209 temperature was fixed at 160 °C for AN. For the analysis of EO, temperature conditions

210 were set as follows: initial temperature of 50 °C for 2 min, increased to 200 °C at 5
211 °C.min⁻¹, then hold for 2 min, giving a total runtime of 34 min.

212

213 *2.8.1. Determination of partition coefficient (K)*

214 The vapor-liquid partition coefficient (*K*) is the ratio of the concentration of a substance
215 in vapor phase to its concentration in liquid phase, when the equilibrium is reached
216 (Kolb and Ettre, 2006).

217

$$K = \frac{C_G}{C_L} \quad (1)$$

218

219 *K* of AN was determined in water and in the different supramolecular mixtures at 30 °C
220 by using the phase ratio variation method for AN in water and the vapor phase
221 calibration method for AN in the supramolecular mixtures as reported earlier (Moura et
222 al., 2017).

223

224 *2.8.2. Retention of AN and essential oils (EO)*

225 The retention of AN, star anise and fennel EO in the formulations was estimated
226 according to procedures developed for aromas (Decock et al., 2008) and EO (Kfoury et
227 al., 2015), respectively. The percentage of retention of AN and EO by the LMM was
228 determined by SH-GC at 30 °C following equation (2) for AN and equation (3) for EO:

229

$$\% \text{ Retention} = \left(1 - \frac{A_{LMM}}{A_W}\right) \times 100 \quad (2)$$

230

231 where A_{LMM} and A_W stand for the peak area of AN in presence of LMM and of water,
232 respectively.

233

$$\% \text{ Retention} = \left(1 - \frac{\Sigma A_{LMM}}{\Sigma A_W} \right) \times 100 \quad (3)$$

234

235 where ΣA_{LMM} and ΣA_W stand for the sum of peak areas of the EO components in
236 presence of LMM and of water, respectively.

237

238 *2.8.3. Multiple Headspace Extraction (MHE)*

239 MHE is a quantitative method, consisting of successive headspace extractions (Kolb
240 and Ettre, 2006). It was used to study the release kinetics of AN from the prepared LMM
241 at 60 °C. Samples were prepared similarly to the previous experiments but, once
242 equilibrium was reached, vials were subjected to 10 headspace extractions at 1-hour
243 intervals.

244

245 **3. Results and Discussion**

246

247 *3.1. Stability studies of LMM by NMR*

248 A recent study showed that ChCl:carboxylic acid DES underwent esterification
249 regardless of the method or the temperature employed during their preparation.
250 However, the DES based on levulinic acid was the least affected, with only 6 mol% of
251 the ChCl esterified at 100 °C (Rodriguez Rodriguez et al., 2019). In the present study,

252 the thermal stability of the new supramolecular mixtures was investigated using ^{13}C
253 NMR, by following the modification of the chemical shift of the carboxylic acid function of
254 levulinic acid. All the supramolecular mixtures were stable after consecutive exposures
255 of 24 hours at 30 and 60 °C. After an additional 24 hours at 80 °C, a small amount of
256 esterification was observed and identified by the presence of a new broad signal around
257 173.0 ppm corresponding to the ester function (Figure S1). No esterification was
258 detected in the case of Captisol®:Lev, which could be due to steric hindrance from the
259 relatively bulky sulfobutyl ether moieties. On the other hand, the most affected LMM
260 was HPBCD:Lev with the relative intensity ratio of the esterified carbon peak being
261 1.6% of the carboxylic acid carbon peak at 100 °C (Table S1) and could be the result of
262 a higher reactivity of the hydroxyl groups, especially that of the hydroxypropyl moieties.
263 Therefore, the preparation of the LMM was performed at 60 °C for all other experiments,
264 in order to avoid esterification. Furthermore, the long-term stability of the LMM was
265 evaluated after 18 months of storage at room temperature. The mixtures showed no
266 degradation over time. The low esterification ratio observed for these CD-based
267 mixtures compared with ChCl-based DES could be explained by the fact that the
268 remaining hydroxyl groups of the CD derivatives used in this study are sterically
269 hindered since the degree of substitution varied from 4.9 to 12.9.

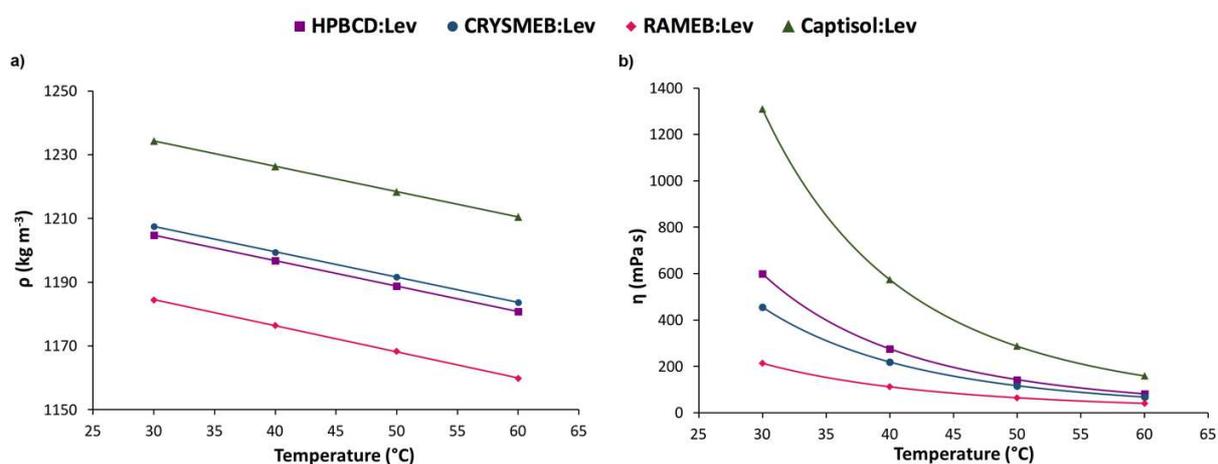
270

271 3.2. Physicochemical characterization

272 The density and viscosity values of the four LMM (HPBCD:Lev, RAMEB:Lev,
273 CRYSMEB:Lev and Captisol®:Lev) were measured from 30 to 60 °C, at 10 °C intervals
274 (Figure 2 and Tables S2 and S3). CD-based mixtures presented higher density values

275 (ranging between 1184.5 and 1234.3 kg m⁻³ at 30 °C) compared with common DES
 276 based on levulinic acid (1134.5 and 1105.7 kg m⁻³ at 303.15 K for ChCl:Lev or
 277 TBPBr:Lev DES respectively) (Moufawad et al., 2019; Moura et al., 2017). Additionally,
 278 the obtained values fall in the 1000-1300 kg m⁻³ range of density values observed with
 279 most of the reported DES (Tang and Row, 2013). LMM were also more viscous than
 280 these common DES, except for RAMEB:Lev which presents similar viscosity to
 281 ChCl:Lev (212.9 and 206.2 mPa.s at 30 °C, respectively).

282



283

284 Figure 2. Experimental values of the density (a) and viscosity (b) of the supramolecular
 285 LMM. The lines represent the Vogel–Fulcher–Tammann (VFT) correlation fitting for
 286 viscosity and appropriate polynomials in the case of the density.

287

288 Nevertheless, the viscosity values decreased remarkably with increasing temperature.
 289 All CD-based mixtures, except Captisol®:Lev, show relatively low viscosities (≤ 80
 290 mPa.s) at 60 °C. The relatively higher viscosity of Captisol®:Lev can be explained by a
 291 stronger hydrogen bond network, given the higher number of HBA sites present in
 292 Captisol® compared to the other studied β -CD derivatives due to the presence of the

293 sulfonate groups. In addition, the CD:Lev mixtures were less viscous than the mixtures
294 based on β -CD derivatives and *N,N'*-dimethylurea. Indeed, the latter mixtures present a
295 melting point above 80 °C and their viscosities were at least equal to 205.0 mPa.s at 90
296 °C (Jérôme et al., 2014).

297 The flow behavior of the four LMM was then investigated at 30 °C. All the mixtures
298 studied exhibit a Newtonian plateau for shear rates below 1000 s⁻¹ and shear-thinning
299 behavior for shear rates above this value (Figure S2). The measured static and dynamic
300 viscosities for the same composition were slightly different (for example, in the case of
301 RAMEB:Lev at 30 °C, $\eta_{\text{static}} = 212.9$ mPa.s vs. $\eta_{\text{dynamic}} = 245.0$ mPa.s) but the same
302 order was found for the viscosities ($\eta_{\text{Captisol@:Lev}} > \eta_{\text{HPBCD:Lev}} > \eta_{\text{CRYSMEB:Lev}} > \eta_{\text{RAMEB:Lev}}$).
303 Moreover, the shear-thinning behavior was more pronounced as the viscosity increased.
304 Indeed, for a high shear rate of 5000 s⁻¹, a decrease of around 29% in viscosity was
305 observed for Captisol@:Lev, whereas in the case of RAMEB:Lev a smaller decrease
306 (around 8%) was detected. As explained above, the most viscous mixtures probably
307 contain a larger number of hydrogen bonds that may be disrupted at high shear rates,
308 leading to a greater decrease in viscosity.

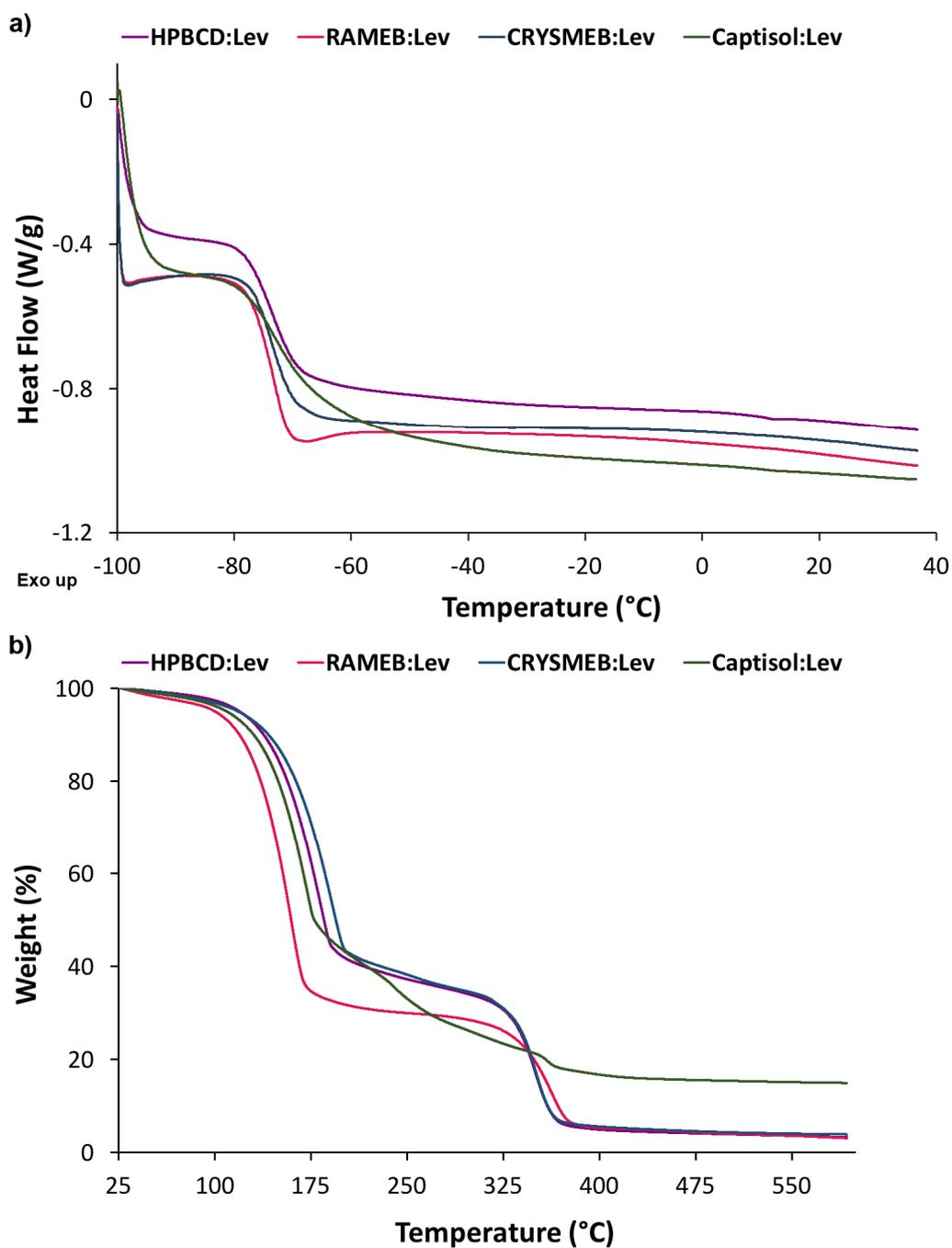
309

310 3.3. *Thermal properties*

311 DSC measurements were performed over a range of -100 to 40 °C in order to
312 understand the thermal events occurring in this temperature range. We chose to focus
313 on the lower temperature range, given that the samples under study were all liquid at
314 room temperature. Interestingly, none of the CD-based mixtures, *i.e.* HPBCD:Lev,
315 RAMEB:Lev, CRYSMEB:Lev and Captisol@:Lev, showed any melting point in the

316 heating curve. Instead, glass transition curves with T_g at -73.3, -74.3, -73.5 and -67.8 °C
317 were observed respectively for the mixtures (Figure 3a). This absence of melting peaks
318 and presence of glass transition temperature was widely observed in the literature. For
319 example, Francisco et al. reported 21 systems showing no melting point but glass
320 transition temperatures ranging between -13.64 and -77.73 °C (Francisco et al., 2012)
321 while Dai et al. identified 13 different NADES with $T_g < -50$ °C and no melting point (Dai
322 et al., 2013). Likewise, with the exception of glass transition, no other thermal events
323 occur for trehalose:glycerol at a 1:30 molar ratio (Castro et al., 2018). Moreover, the
324 observed T_g value of -75.14 °C is interestingly pretty close to the values obtained for our
325 CD-based mixtures. CD generally present a T_g ranging between 80 °C and more than
326 200°C depending on the CD (Tabary et al., 2011). Therefore, the observed T_g values
327 could be attributed to the formation of a LMM with levulinic acid.

328



329

330 Figure 3. DSC (a) and thermogravimetric analysis (b) curves of the LMM.

331

332 Dynamic thermogravimetric analysis (TGA) was used to further investigate the thermal
 333 stability of the new mixtures. As shown in Figure 3b, they underwent progressive

334 decomposition as the temperature increased. Their decomposition showed a two-step
335 weight loss similar to choline-based DES (Delgado-Mellado et al., 2018). At around 130
336 °C, levulinic acid began to decompose while the CD began to degrade at about 325 °C,
337 except in the case of Captisol® for which the second decomposition began at around
338 225 °C and a third decomposition was determined near 350 °C. From the
339 thermogravimetric curve in Figure 3b, the results showed that the thermal
340 decomposition temperatures corresponding to the first thermal event of HPBCD:Lev,
341 RAMEB:Lev, CRYSMEB:Lev and Captisol®:Lev were equal to 130.4, 117.6, 137.7 and
342 127.6 °C respectively. CRYSMEB:Lev had the best thermal stability and RAMEB:Lev
343 the worst (Table S3). The DSC and TGA results demonstrate that these CD-based
344 mixtures retain a stable liquid state over a wide temperature range.

345

346 3.4. Solubilization of AN and essential oils

347 AN has incited interest from the food, cosmetics and pharmaceutical industries due to
348 its attractive properties. In fact, AN is a major component of star anise and fennel
349 essential oils which are known both as flavoring agents and as medicines owing to their
350 numerous biological properties (Auezova et al., 2020; Diao et al., 2014; Wang et al.,
351 2018). However, its wider applications are hampered by drawbacks related to its high
352 volatility, low water solubility and chemical instability. We have previously investigated
353 the effect of AN complexation with various CD on its solubility and photodegradation
354 and showed that CD were able to improve AN solubility and stability through inclusion
355 complex formation (Kfoury et al., 2014a, 2014b). Therefore, the ability of the LMM to
356 solubilize AN was evaluated by determining the vapor-liquid partition coefficient K of AN

357 (Table 2) as well as the percentage of AN retention. More than 99% of AN were
 358 retained by all the studied mixtures while in aqueous solution CD were able to reduce
 359 AN volatility only up to 92% leading to around 20-fold reduction in K (Kfoury et al.,
 360 2014b, 2014a). In the case of the supramolecular mixtures we observed up to 1300
 361 times reduction in K compared with water (Table 2).

362

363 Table 2. Partition coefficient (K) values of *trans*-anethole in water and in the tested
 364 mixtures and ratio of $K_{\text{water}}/K_{\text{LMM}}$ at 30°C

365

	K	Ratio
Water	1.29×10^{-2}	1
HPBCD:Lev	1.04×10^{-5}	1240
RAMEB:Lev	9.60×10^{-6}	1343
CRYSMEB:Lev	1.08×10^{-5}	1194
Captisol®:Lev	2.18×10^{-5}	592

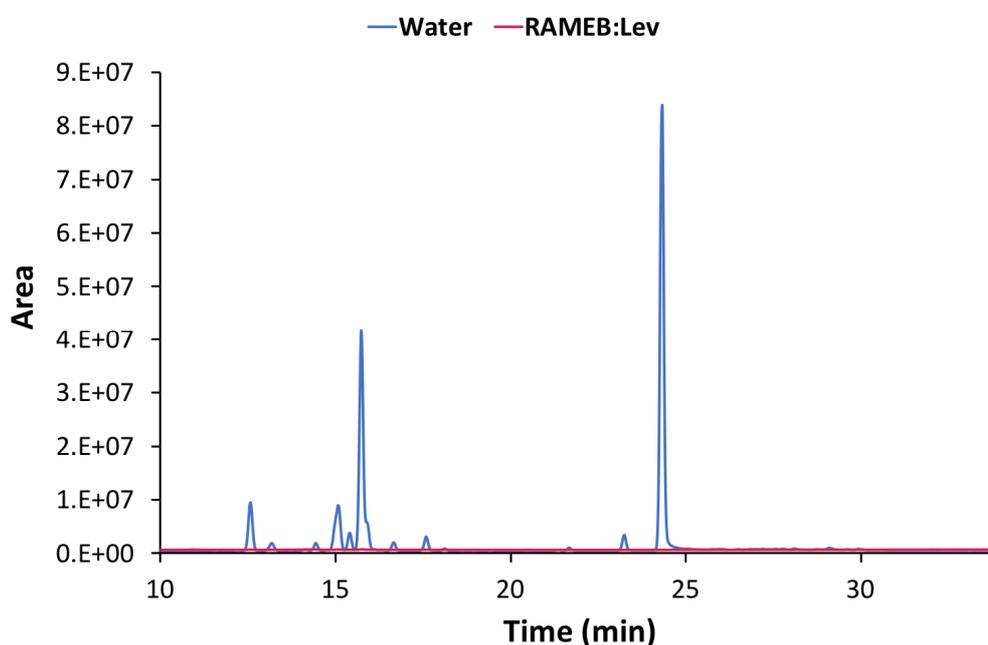
366

367 DES have been explored as solvents for the extraction of EO or their components in the
 368 past few years (Li et al., 2019; Ozturk et al., 2018), with higher extraction yields
 369 observed compared with conventional solvents. However, the majority of the reported
 370 studies used diluted DES to improve their extraction efficiency by reducing their
 371 viscosity or increasing their polarity (Jeong et al., 2018). To the best of our knowledge,
 372 no studies investigating the solubilization or the extraction of AN or star anise EO using
 373 DES have been performed to date. Only one publication has reported the use of lactic
 374 acid-based NADES for the extraction of polyphenols from fennel EO, among other

375 Greek medicinal plants (Bakirtzi et al., 2016). Subsequently, the ability of the studied
376 LMM to solubilize EO, as well as AN in the presence of other aroma compounds, in two
377 EO was investigated.

378 Star anise and fennel EO were chosen for having AN as a major component. All the
379 tested mixtures showed high retention ability ($\geq 98.9\%$) toward star anise and fennel EO
380 (Figure S3). Figure 4 depicts the chromatogram of star anise EO obtained by SH-GC in
381 presence of RAMEB:Lev and in presence of water as a reference solvent. Clearly, the
382 peaks corresponding to the volatile compounds found in star anise are obvious in water
383 but almost disappear in presence of the CD-based mixture. Figure S4 shows the
384 chromatograms of star anise EO in the three other mixtures.

385



386

387 Figure 4. Chromatogram of star anise essential oil in water and in RAMEB:Lev.

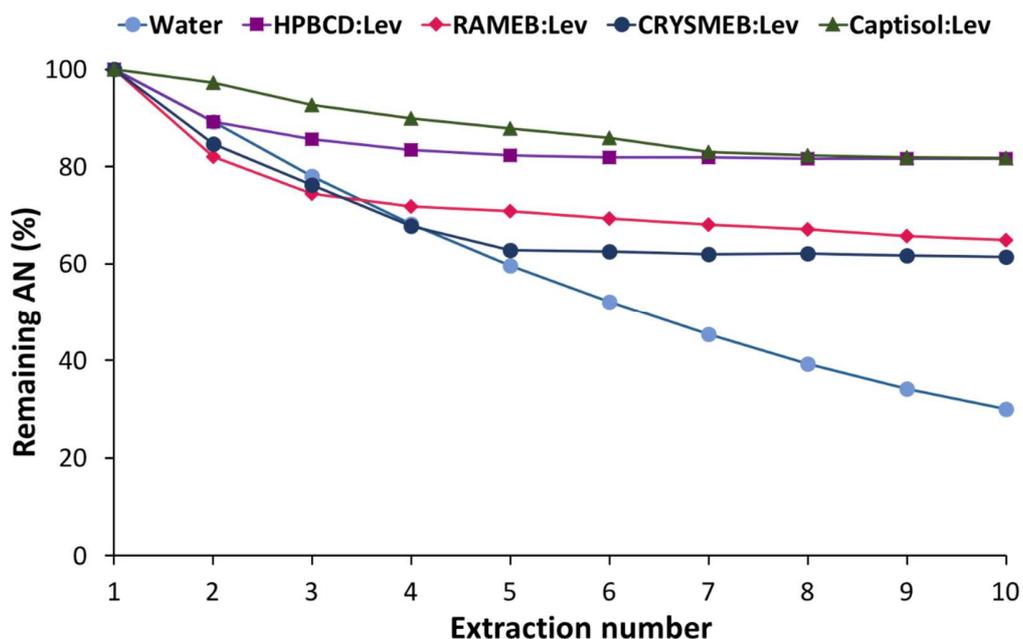
388

389 Concerning the retention of AN, similar results were obtained regardless of whether AN
390 was added in pure form or as a constituent of star anise or fennel EO (Figure S5).
391 Therefore, the ability of the mixtures to retain AN was not affected by the simultaneous
392 presence of other compounds.

393

394 3.5. Release study

395 Finally, the release of AN from the LMM was monitored by MHE. As described above,
396 10 successive headspace extractions were conducted at one-hour intervals. The
397 amount of AN present in the vial after each extraction could be determined using the
398 peak area. The retention ability of the LMM can be monitored and compared to water.
399 The release profiles of AN from LMM and water are shown in Fig. 5. The results indicate
400 that these CD-based mixtures can not only solubilize AN but also delay its release over
401 time. Indeed, 70% of AN was released from water after 10 extractions at 60 °C, while
402 only 20-40% was released from the CD:Lev mixtures.



403

404 Figure 5. Release of *trans*-anethole from water and from supramolecular mixtures.

405

406 **4. Conclusions**

407 New supramolecular mixtures based on β -CD derivatives and levulinic acid were
408 prepared, characterized and studied for their ability to solubilize AN and related
409 essential oils. In addition, their thermal stability was investigated. The physicochemical
410 characterization studies demonstrated that these CD-based mixtures are liquid at room
411 temperature with relatively low viscosity. The stability studies also revealed that they
412 were stable up to 60 °C and that no degradation could be detected after 18 months of
413 storage. These mixtures also presented high solubilizing ability towards AN, star anise
414 and fennel essential oils, when compared with water or aqueous solutions of CD.
415 Furthermore, they were able to delay the release of AN, as shown by MHE experiments.
416 When compared to aqueous CD solutions, these mixtures guarantee the presence of
417 high amounts of CD associated to a liquid state over a wide temperature range, which
418 consequently allow the broadening of CD applications. These mixtures may therefore
419 contribute in the search for greener solvents and result in new promising, safe and
420 economic drug delivery systems. Indeed, due to their peculiar properties (viscosity,
421 stability and encapsulation properties), these supramolecular LMM could solubilize
422 poorly water-soluble drugs and/or protect fragile molecules during topical cutaneous
423 administration. The potential of these LMM is currently being tested for the treatment of
424 cutaneous leishmaniasis with a volatile and poorly water-soluble anti leishmaniasis drug
425 and the results will be communicated in due course.

426

427 **Acknowledgements**

428 The authors thank the Research Funding Program at the Lebanese University,
429 Lebanon, and the Agence Universitaire de la Francophonie, Moyen-Orient (PCSI 2018-
430 2020) for funding the project. Tracy El Achkar is grateful to the Université du Littoral
431 Côte d'Opale and the Lebanese University for providing her a scholarship. Fanny
432 SIMELIERE from Institut Galien Paris-Sud (Châtenay-Malabry, France) and Jacques
433 LOUBENS from TA Instruments (Guyancourt, France) are greatly acknowledged for
434 their generous help. This work partially benefited from the support of the project
435 ParasiDES ANR-19-CE18-0027 of the French National Research Agency (ANR).

436

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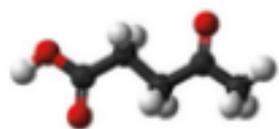
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Modified β -cyclodextrins

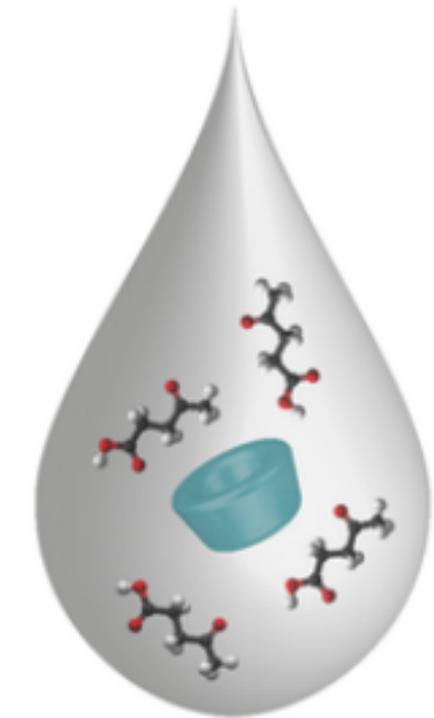


mp > 280 °C

+

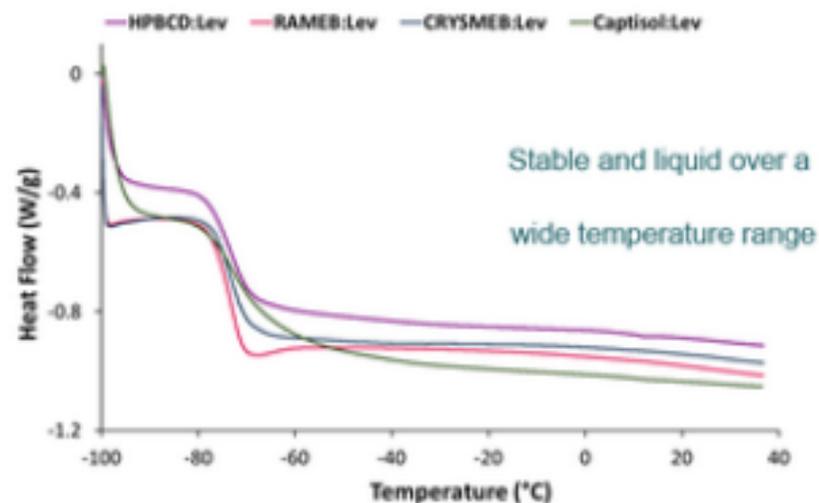


Levulinic acid



New liquid mixtures
based on cyclodextrins

Physicochemical
characterization



Solubilization of
bioactive compounds

